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Insulin-Like Growth Factor (IGF)-I and -II and IGF-Binding Proteins-1, -2, and -3 in Children and Adolescents with Diabetes Mellitus: Correlation with Metabolic Control and Height Attainment*

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ABSTRACT

The putative effects of diabetes and metabolic control on circulating levels of insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) remain controversial. In the present study, serum levels of IGF-I and IGF-II and IGFBP-1, -2, and -3 were measured in 58 patients (age, 0.8–17 yr) with treated (51 subjects) or untreated (7 subjects) insulin-dependent diabetes mellitus (IDDM) and were compared with the levels in normal subjects. In the untreated patients IGF-I and IGF-II were decreased as compared with the healthy controls. In the treated diabetics IGF-I and IGF-II were reduced; IGFBP-2 (only in prepubertal subjects) and IGFBP-3 were increased. Furthermore, age-adjusted values of IGF-I, IGF-II, and IGFBP-3 were lower in prepubertal than in pubertal patients. Regression analysis revealed a negative correlation between hemoglobin (Hb)A1c and standard deviation scores (SDS) of IGF-I and a positive association between HbA1c and IGFBP-1 SDS or IGFBP-2 SDS. In the treated patients HbA1c was positively related to IGFBP-1 SDS and IGFBP-2 SDS when applying simple regression analysis and to IGFBP-2 SDS when using a multiple regression model. Strong correlations were observed between height SDS and IGF-I SDS, IGF-II SDS, and IGFBP-3 SDS in prepubertal subjects who had had IDDM for at least 2 yr, but not in adolescents. Such correlations have also been found in healthy children and adolescents. In conclusion; 1) IDDM is associated with alterations of the IGF-IGFBP system, which are partially accounted for by differences in metabolic control and pubertal status; 2) the lower plasma concentrations of serum IGF-I may play a role in the pathogenesis of growth impairment of poorly controlled prepubertal, but not pubertal, children and adolescents with IDDM; and 3) in addition, a potential role of the altered IGF-IGFBP system for the development of diabetic late complications is hypothesized. (*J Clin Endocrinol Metab* **80:** 1207–1213, 1995).

SHORT- and long-term consequences of insulin-dependent diabetes mellitus (IDDM) not only result from insulin deficiency itself but also from secondary metabolic and hormonal derangements. In the last decade interest has focused on insulin-like growth factor-I (IGF-I) as a candidate molecule that may play a key role in the pathogenesis of immediate or late diabetic complications (1–7).

IGF-I is a GH-dependent polypeptide that has a 3-fold function as a mediator of the growth-promoting action of GH, as a potent mitogenic factor, and as a metabolic regulator with insulin-like activity (1, 7). Alterations of IGF-I regulation may provide an attractive explanation for IDDM-associated growth impairment as well as for proliferative microvascular complications. So far, it is unclear whether IGF-II, a peptide that is homologous to IGF-I, could likewise play a pathophysiological role in diabetic complications. *In vivo* and *in vitro* studies have suggested mitogenic and metabolic functions for IGF-II that are similar to those of IGF-I. In addition, IGF-II is thought to be an important regulator of fetal and embryonic growth (2, 7). It is unclear whether IGF-II is GH dependent (1, 2, 7). Unlike IGF-I, the serum concentration of which increases during childhood and puberty secondary to rising GH secretion, in the human IGF-II levels remain nearly constant after an initial rise during the first year of life (7–9). In the circulation the vast majority of the IGFs is bound to specific carrier proteins, the IGF-binding proteins (IGFBPs), which greatly prolong their half-time and regulate their bioavailability and their bioactivity (3, 4, 10, 11). So far, six different IGFBPs (IGFBP-1 to -6) have been cloned and sequenced.

The predominant IGFBP in the blood is IGFBP-3, which forms a large 150-kilodalton (kDa) ternary complex. The serum level of this complex determines the total concentration of circulating IGFs and regulates the growth-promoting potential of IGF-I (2, 10–19). Serum levels of IGF-I and IGFBP-3 are positively related to nutritional status and age during childhood and adolescence (20–25). It is not

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definitively settled, however, whether IGFBP-3 is acutely regulated by GH or IGF-I (26–28). Current oppinion favors GH as the major regulator of IGFBP-3 levels in the human. *In vivo* and *in vitro* experiments indicate relatively poor control of IGFBP-3 concentrations by IGF-I (8, 9, 20, 29–31).

The second most abundant IGFBP in the circulation is IGFBP-2, a 31.3-kDa protein with a preferential affinity for IGF-II. Little is known about the regulation and biological function of IGFBP-2. In animals insulin may control IGFBP-2 production (21, 23). However, no such regulation was found in man (20).

IGFBP-1, a 25-kDa carrier protein, might play an important role in modulating mitogenic and metabolic effects of free IGFs. Insulin-like activity is blocked by excess IGFBP-1, whereas the mitogenic potential of the IGFs can be enhanced or inhibited by IGFBP-1 depending on the target cells and the experimental conditions (14–17). Inasmuch as hepatic IGFBP-1 secretion is directly suppressed by insulin and stimulated by substrate deprivation (18, 19, 24–28, 32), this carrier protein might be directly involved in glucose counterregulation. IGFBP-1 might inhibit the glucose-lowering potential of IGFs in response to hypoglycemia and allow for the expression of insulin-like activity after food intake. IGFBP-1 levels are inversely related to insulin levels in healthy (25, 26) and in diabetic subjects (27, 28). They decline with advancing age, coinciding with rising insulin secretion (32, 33). A marked circadian variation with peak levels in the early morning when insulin levels are lowest has been reported (32).

The close relationship between the IGF-IGFBP system and glucose homeostasis has encouraged many investigators to study the influence of diabetes on the serum levels of the IGFs and/or the IGFBPs. The data from these studies are rather conflicting. We have systematically and simultaneously measured serum levels of five components of the IGF-IGFBP system in children and adolescents with diabetes. Importantly, we have asked whether alterations of the IGF-IGFBP system impair growth of diabetic children.

Subjects and Methods

Diabetic subjects

The study population consisted of 58 children and adolescents with IDDM, 21 girls and 37 boys. At the time of blood sampling 7 patients had not yet received exogenous insulin therapy, since they had just been hospitalized with newly detected diabetes; none of them had required intensive care hospitalization. The remaining 51 patients had been diabetic for at least 3 months before blood sampling. Insulin therapy was conventional (2 daily injections) in 40 and intensified (at least 3 daily injections) in 11 subjects. Apart from IDDM, none of the patients had an acute or chronic illness. Other than insulin the patients did not receive any medication. No hormonal deficiency that could be expected to interfere with metabolism of IGFs or IGFBPs was present in any of the patients. The patients' heights, weights, pubertal stages according to Tanner, and HbA1c values were recorded at each clinical visit. Hemoglobin A1c (HbA1c) was measured by high performance liquid chromatography (Diamat, BioRad, Richmond, CA). Venous blood samples for determination of IGFs and IGFBPs were obtained during routine visits in the afternoon together with samples for routine blood testing. The study protocol was approved by the Ethical Committee of the Children's Hospital, University of Munich. Parents and patients gave their informed consent.

Control subjects

The control population consisted of 600 healthy, male and female children and adolescents. Serum samples for measurement of the IGFs and IGFBPs were collected during daytime. The results were used to establish percentiles for each biochemical parameter. Comparisons between the patients and the reference subjects were based on age-independent standard deviation scores (SDS) (29, 34, 35).

Biochemical methods

IGF-I RIA. IGF-I was measured by an IGFBP-blocked RIA using a highly specific polyclonal antiserum with a cross-reactivity with IGF-II of less than 0.05% (a kind gift of Drs. B. Breier and P. Gluckman, Children's Hospital, University of Auckland, Auckland, New Zealand) (30, 36). Briefly, serum samples were diluted 1:150 in an acidic buffer to dissociate IGFs from IGFBP. On neutralization, a large excess of IGF-II (Mediagnost, Tübingen, Germany) was added to block IGFBP-binding sites. Details of the assay will be published elsewhere. The sensitivity at a serum dilution of 1:150 was 3 μg/L, and the intraassay and interassay coefficients of variation at 50% B/B_0 were 3.2% and 7.4%, respectively.

IGF-II RIA. The assay was performed in acid-ethanol extracts using a specific antiserum for the C-peptide domain of IGF-II (29, 30, 35). Cross-reactivity of the antiserum with IGF-I is less than 0.005%. Interference of residual IGFBPs was blocked by addition of excess IGF-I as described before (35, 36). Intraassay and interassay coefficients of variation at 50% B/B_0 (n = 20) were 3.7 and 9.6%, respectively.

IGFBP RIAs. IGFBP-1 was measured by a specific RIA as described previously (30). IGFBP-2 was measured by RIA using a polyclonal antiserum against a synthetic partial sequence of IGFBP-2 (176–190) as described before (22), except that for preparation of radiolabeled tracer and of standards, recombinant IGFBP-2 (a kind gift of Sandoz, Basel, Switzerland) was used. IGFBP-3 was measured by RIA using a specific polyclonal antiserum against authentic IGFBP-3 purified from human Cohn fraction IV, which showed no cross-reactivity with IGFBP-1 or IGFBP-2 up to 1 mg/L. Details of the assay have been reported previously (37).

Statistics

Results are expressed as mean ± SD, median and range. SDS were used if required to remove an obvious or possible age dependence (height and serum levels of IGFs and IGFBPs). For calculating height z scores the data from the Zurich longitudinal study were used (38). Statistical calculations were performed with the StatWorks program (Cricket Software, Philadelphia, PA). Differences between groups were evaluated by the unpaired *t* test. Simple regression analysis was used to assess the relationship between two parameters. Multiple regression analysis was performed to determine which variables were independently associated with constants. Two-way analysis of variance was applied to both regression analysis models.

Results

Clinical results

Clinical and diabetes characteristics of the study population are presented in Table 1. Actual age, age at onset of diabetes and body mass index (BMI) did not differ between treated and untreated patients. Height SDS and HbA1c were significantly higher in the untreated patients (height SDS, 0.32 *vs.* 0.53, $P < 0.001$; HbA1c, 8.1% *vs.* 12.2%, $P < 0.0001$). When the patients on insulin therapy were grouped as prepubertal (n = 28) and pubertal (n = 23), both groups differed significantly with respect to the duration of diabetes ($P < 0.003$) but not with regard to height SDS, age at onset of diabetes, and HbA1c.

TABLE 1. Clinical and diabetes characteristics of the study subjects

	Mean ± SD	Median	Range
Age (yr)	10.9 ± 3.9	11.0	0.8–17.5
SDS height	−0.23 ± 1.14	−0.17	−2.47–+2.9
Duration (yr)	4.0 ± 3.9	2.9	0–15
Age at onset (yr)	6.9 ± 3.8	6.6	0.8–14.4
Insulin (U/kg)[a]	0.75 ± 0.22	0.78	0.32–1.23
HbA1c (%)	8.6 ± 2.1	8.0	5.2–16.8

[a] Only treated patients (n = 51).

Biochemical results

Serum levels and SDS of IGF-I, IGF-II, and IGFBP-1, -2, and -3 of treated and untreated patients and *P* values for differences between the diabetic patients and the reference population are presented in Tables 2 and 3. Figure 1 shows the graphical distribution of the patients' data within the corresponding percentiles. In the whole group of treated and untreated patients, IGF-I and IGF-II levels were significantly reduced ($P < 0.001$) and serum IGFBP-3 elevated ($P < 0.001$) compared with the reference values. Subjects with untreated IDDM had decreased serum concentrations of IGF-I and IGF-II ($P < 0.01$). IGFBP-2 tended to be increased and IGFBP-3 to be reduced in subjects with untreated IDDM. These differences were not significant due to great interindividual variation. In the group of patients with treated IDDM, a marked reduction of IGF-I and IGF-II levels ($P < 0.001$) and an increase of IGFBP-3 levels ($P < 0.001$) was evident. Comparison of patients with and without insulin therapy revealed lower serum concentrations of IGF-I and IGFBP-3 ($P < 0.0001$) and higher IGFBP-2 levels ($P < 0.0001$) in the untreated diabetics. When the population of treated patients was grouped into prepubertal and pubertal, serum levels of IGF-I ($P < 0.01$) and IGFBP-3 ($P < 0.03$) were lower in the prepubertal patients than in the pubertal patients (IGF-1 SDS, −0.86 *vs.* −0.21; IGFBP-3 SDS, 0.84 *vs.* 1.47). Serum concentrations of IGF-I were normal in the pubertal patients but reduced ($P < 0.001$) in the prepubertal patients. IGFBP-2 levels were normal in the pubertal patients but elevated in prepubertal patients ($P < 0.01$). Serum IGF-II was reduced ($P < 0.001$) and serum IGFBP-3 increased ($P < 0.001$) both in pubertal and prepubertal patients.

Possible interrelationships between clinical or diabetes parameters (age, BMI, insulin dose, duration of diabetes, age at onset, and HbA1c) and the SDS of the IGFs and IGFBPs were assessed by simple regression analysis. The analysis was applied to the overall study population and also to the group of treated patients alone. The results of this analysis are presented in Table 4. Figure 2 shows the relationship between IGF-I SDS or IGFBP-2 SDS and HbA1c in the overall study population; IGFBP-2 and IGFBP-1 levels correlate significantly with HbA1c levels, whereas IGF-I is inversely related to HbA1c. Multiple regression analysis was performed to evaluate independence of variables (gender, age, BMI, duration of diabetes, and HbA1c). Parameters without significant correlations in the simple regression model were not included in this analysis. Possible relationships between SDS height and the SDS of the IGFs and IGFBPs were evaluated only in those patients who had IDDM for at least 2 yr. In this population, simple regression analysis revealed positive correlations between height SDS and IGF-I SDS ($r = 0.47$; $P < 0.004$), IGF-II SDS ($r = 0.43$; $P < 0.008$), and IGFBP-3 SDS ($r = 0.53$; $P < 0.001$). Analogous results were obtained with multiple regression analysis using gender, age, duration of diabetes, and HbA1c as covariables (Table 5). In the prepubertal subjects, however, height SDS was strongly related to IGF-I SDS ($F = 4.4$; $r = 0.76$; $P < 0.0001$), IGF-II SDS ($F = 5.3$; $r = 0.71$: $P < 0.0001$), and IGFBP-3 SDS ($F = 5.1$; $r = 0.8$; $P < 0.0001$). Such correlations were not found in pubertal patients. It is important to realize that height SDS did not differ significantly between both groups of patients (−0.37 in prepubertal *vs.* −0.13 in pubertal patients).

Discussion

This study reveals the presence of multiple alterations in the IGF and IGFBP system of diabetic children and adolescents. IGF-I levels were reduced in patients with and without insulin therapy. In the literature, data regarding IDDM-associated alterations of IGF-I levels are conflicting (5, 39–46). In adult patients IGF-I has been demonstrated to be decreased (33, 44, 45, 47, 48), normal (6, 27) or increased (5, 44). When IGF-I levels in children and adolescents are measured, the strong age dependence of IGF-I should be taken into account. Of three studies fulfilling this criterion, one found decreased serum IGF-I in well controlled patients (43); another reported subnormal IGF-I levels in adolescents (42); and one found no significant difference of IGF-I levels between diabetics and healthy children and adolescents (40). In none of the studies were age-adjusted IGF-I values of prepubertal and pubertal patients compared. As a reason why IGF-I levels are normal in our adolescent diabetics but low in the prepubertal patients, the following possible explanations come to mind. The diabetes-associated suppression of IGF-I generation is attributed to GH receptor (6, 49) or postreceptor (45, 46) defects. Although GH is the major determinant of circulating IGF-I, other factors such as sex steroids

TABLE 2. Serum levels and SDS of IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 in 51 treated patients with IDDM

	Serum levels (μg/L)			SDS			*P*[a]
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
IGF-I	174 ± 98	140	25–496	−0.57 ± 1.03	−0.55	−3.34–+1.63	<0.001
IGF-II	489 ± 120	479	166–777	−1.06 ± 1.15	−0.8	−4.48–+1.13	<0.001
IGFBP-1	30.6 ± 16.7	25	14–98	−0.15 ± 0.57	−0.23	−1.27–+1.62	NS
IGFBP-2	381 ± 141	356	166–689	0.27 ± 1.49	0.38	−2.4–+3.34	NS
IGFBP-3	3868 ± 882	3964	1302–5772	1.12 ± 0.98	1.28	−2.24–+2.61	<0.001

[a] The *P* values refer to the difference between the patients and the control group, calculated on the basis of SDS.

TABLE 3. Serum levels and SDS of IGF-I, IGF-II, IGFBP-1, IGBBP-2, and IGFBP-3 in seven untreated patients with IDDM

	Serum levels (μg/L)			SDS			P^a
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
IGF-I	75 ± 41	78	24–139	−3.68 ± 2.41	−2.9	−7.6–−0.55	<0.01
IGF-II	443 ± 185	439	184–654	−2.09 ± 2.5	−1.97	−6.81–+0.35	<0.01
IGFBP-1	36.1 ± 17.5	34	14–72	0.15 ± 0.59	1.4	−0.54–+1.3	NS
IGFBP-2	732 ± 737	437.5	240–2357	2.34 ± 3.47	1.77	−1.42–+8.9	NS
IGFBP-3	2761 ± 13282	2751	828–4609	−1.20 ± 2.99	−0.78	−7.03–+2.01	NS

[a] The P values refer to the difference between the patients and the control group, calculated on the basis of SDS.

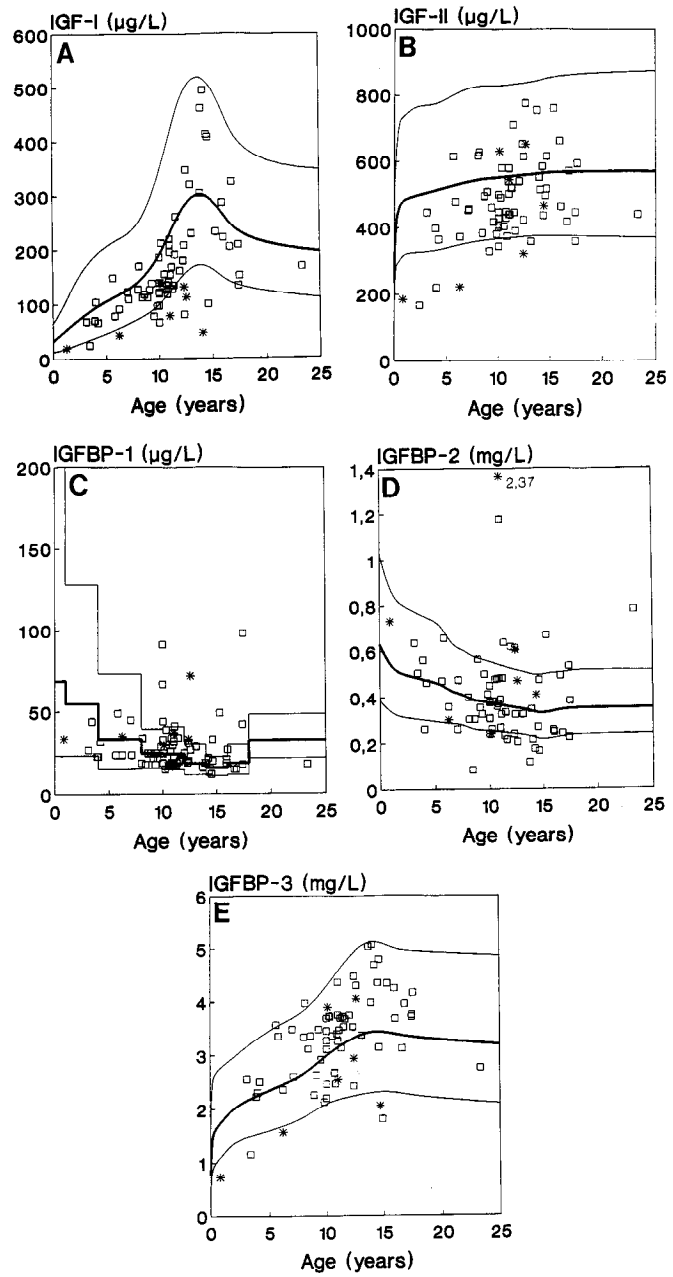

FIG. 1. Serum levels of IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 in 58 patients with IDDM. The plots depict IGF-I (a), IGF-II (b), IGFBP-1 (c), IGFBP-2 (d), and IGFBP-3 (e) levels according to age (in years). The *lines* give the 5th, 50th, and 95th percentiles. *Symbols* are for individual patients: *stars*, untreated patients; *boxes*, treated patients.

TABLE 4. Correlations between the SDS of IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, and clinical parameters as evaluated by simple regression analysis

Constant	Variable	All patients (n = 58)		Treated patients (n = 51)	
		r	P	r	P
IGF-I SDS	HbA1c (%)	−0.46	<0.0001		
IGF-II SDS	Age (yr)	0.34	<0.01		
IGFBP-1 SDS	Age (yr)	0.43	<0.001		
IGFBP-1 SDS	Duration (yr)	0.37	<0.005	0.31	<0.03
IGFBP-1 SDS	HbA1c (%)	0.46	<0.0001	0.32	<0.03
IGFBP-2 SDS	HbA1c (%)	0.38	<0.003	0.3	<0.04
IGFBP-3 SDS	Age (yr)	0.36	<0.01		

Only significant correlations ($P < 0.05$) are depicted.

also stimulate IGF-I secretion (50–52). Thus, the puberty-associated increment of sex hormone serum concentrations could lead to the normalization of lowered IGF-I concentrations during puberty. Since diabetes may result in delayed puberty, it is plausible that IGF-I levels that are lower in diabetic children than in controls would be normal when adjusted for pubertal stage rather than for age. However, no obvious pubertal delay was present in our patients.

Several investigators reported a significant increase of IGF-I levels on improvement of metabolic control in children and adolescents with IDDM (5, 6, 40, 44, 47, 53). These data suggest a possible effect of glycemic control on IGF-I serum levels (40, 47). Indeed, we could demonstrate a significant inverse correlation between HbA1c and SDS IGF-I in our study population. In addition, in five of our patients serial IGF measurements could be performed over a 2-yr period: although the number of patients was too small to allow for mathematical calculations, IGF-I SDS scores rose when metabolic control improved in three patients; in one patient IGF-I serum levels remained constant despite improved metabolic control, and in one patient both IGF-I SDS and HbA1c remained stable (data not shown). These findings, along with the data from other studies (5, 6, 39, 40, 44, 47, 53), suggest a possible effect of glycemic control on IGF-I levels in poorly controlled diabetic children.

Since IGF-I plays a major role in growth regulation, we were interested in the relationship between height and IGF-I values. We found a strong association between height SDS and IGF-I SDS in prepubertal patients but not in pubertal patients. This is in agreement with a study from Dacou-Voutetakis *et al.* (54), who found no significant correlation of IGF-I levels with height SDS in healthy pubertal subjects. In healthy children and adolescents, a steeper regression line between IGF-I and GH secretory capacity is found during puberty than before sexual maturation. As in our diabetic

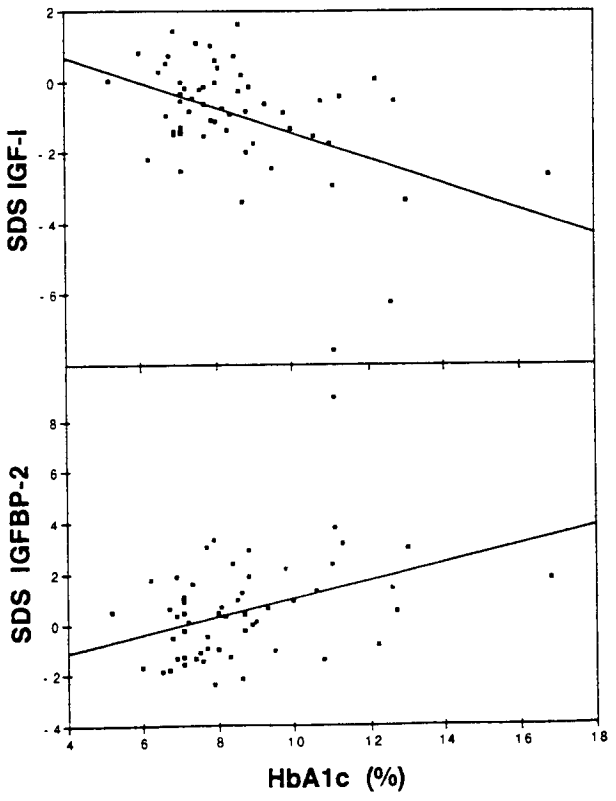

FIG. 2. Relationship between HbA1c and SDS IGF-I and HbA1c and SDS IGFBP-2 in 58 patients with IDDM (SDS IGF-I *vs.* HbA1c, r = −0.46; $P < 0.0001$; SDS IGFBP-2 *vs.* HbA1c, r = 0.38; $P < 0.003$).

patients, IGF-I was highly correlated with height SDS in prepubertal healthy children (37). Thus, IGF-I and height SDS show a significant correlation in patients with IDDM similar to the correlation that exists in healthy children and adolescents.

Little data exist regarding the influence of IDDM on IGF-II serum levels. Normal IGF-II values have been reported in small series of diabetic adults (5) and adolescents (39). Nevertheless, in the study of Amiel *et al.* (39), four patients with very low serum IGF-I also had depressed IGF-II levels, which were normalized with improved metabolic control. We found a significant reduction of IGF-II levels in treated as well as in untreated IDDM patients.

Since IGFBP-1 seems to be the only component of the IGF-IGFBP system that is directly regulated by insulin, its pathophysiological role in diabetes is an area of intense investigation. When considering only studies with adequate age-matching or age-corresponding reference values, a significant increase of serum IGFBP-1 in adults and adolescents with IDDM is reported consistently (27, 28, 33, 48, 55, 56). In contrast, we did not find any significant alteration of IGFBP-1 levels in our patients. The reason for this discrepancy is not clear.

Although mean HbA1c values of our patients were much lower than those reported in most previous studies, differences in glycemic control cannot explain our unexpected findings, since we could not demonstrate markedly elevated serum IGFBP-1 levels even in patients with newly diagnosed, untreated IDDM. The serum samples of our study and control populations were collected under nonstandardized conditions. An optimal study design had to take into account that IGFBP-1 levels vary depending on daytime and food intake. However, substantial fluctuations of serum IGFBP-1 are mainly demonstrable during the night, whereas the variations during daytime and after meals are rather small (56). The age-adjusted IGFBP-1 values of our treated patients were positively related to the duration of IDDM. Since the mean diabetes duration was rather short (4.5 yr), a longer diabetes duration in other study populations could provide an explanation for the higher IGFBP-1 values reported in the literature. Despite the normal IGFBP-1 levels, we found a significant inverse relationship between SDS IGFBP-1 and HbA1c. This finding is in accordance with previous studies (33, 48, 57). The association between elevated IGFBP-1 levels and IGF-I inhibitory activity on cartilage sulfation in poorly controlled diabetic adolescents has led to the suggestion that IGFBP-1 may play a role in diabetes-related growth impairment (58). However, our study failed to demonstrate a significant relationship between height SDS and IGFBP-1 SDS. In agreement with the literature (48), our study demonstrated a complete abolition of the normal age dependence of IGFBP-1 levels in diabetic patients. This finding is not surprising, since the physiological decrease of serum IGFBP-1 during childhood and puberty results from increasing insulin secretion. In our study population the insulin dose per kilogram did not differ between prepubertal and pubertal patients.

In the group of insulin-treated patients, serum IGFBP-2 was normal in pubertal and elevated in prepubertal subjects ($P > 0.01$). Comparison of patients with and without therapy revealed significantly higher IGFBP-2 levels in the untreated subjects. Interestingly, IGFBP-2 SDS was positively related to HbA1c. This finding would be consistent with a direct or indirect regulation of IGFBP-2 by insulin, analogous to the insulin dependence of IGFBP-1, a hypothesis that is supported by results from animal experiments (21). In contrast, dynamic studies in man rather point to a role of free IGF-I (23) or IGF-II (11, 12, 23, 29) as a major regulator of circulating IGFBP-2. Although IGFBP-3 is not involved in glucose homeostasis, marked alterations of IGFBP-3 levels have been demonstrated in patients with IDDM. Baxter *et al.* (59) reported a 40% reduction of serum IGFBP-3 in 10 adults with insufficient metabolic control. Other studies revealed decreased IGFBP-3 levels in diabetic adolescents (42, 48). In

TABLE 5. Correlations between the SDS of IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, and clinical parameters as evaluated by multiple regression analysis

Constant	Variable	All patients (n = 58)			Treated patients (n = 51)		
		F	r	P	F	r	P
IGF-I SDS	HbA1c (%)	5	0.46	<0.0001			
IGF-I SDS	Height SDS				5	0.47	<0.004
IGF-II SDS	Height SDS				4.5	0.43	<0.008
IGFBP-3 SDS	Height SDS				5	0.53	<0.001
IGFBP-1 SDS	Duration (yr)				5.4	0.51	<0.04
IGFBP-1 SDS	HbA1c (%)	5.8	0.37	<0.001			
IGFBP-2 SDS	HbA1c (%)	2.7	0.38	<0.02	1.5	0.29	<0.03

Only parameters with significant correlations in the simple regression model are included in the analysis.

contrast, we found a significant elevation of serum IGFBP-3 in our treated, fairly well controlled patients. Only the 7 subjects without insulin therapy had a tendency toward reduced IGFBP-3 levels. Although these data could point to an influence of glycemic control on IGFBP-3 levels, no relationship between HbA1c and IGFBP-3 SDS was found. In contrast to the data of Batch *et al.* (48) and in agreement with the findings in Dunger *et al.*'s study population (42) and in healthy subjects, our study revealed a positive relationship between IGFBP-3 levels and chronological age. A strong positive correlation between IGF-I and IGFBP-3 (data not shown) was present both in healthy subjects (37) and in IDDM patients.

It is unclear which of the alterations of the IGF-IGFBP axis that are found in diabetes patients are of clinical significance. Although the influence of iuvenile-onset IDDM on adult height is controversial, most data indicate a negative effect of diabetes on linear growth (60, 61). Intensification of insulin treatment resulting in decreased HbA1c values is not only associated with a significant increase in serum IGF-I, but also with a marked acceleration of growth velocity (6, 53). In our study, mean height SDS was within the normal range. Nevertheless, we found a strong correlation between height SDS and the SDS of IGF-I, IGF-II, and IGFBP-3 in prepubertal patients with a diabetes duration of more than 2 yr. No such correlation existed in adolescents. This finding suggests that circulating IGF-I may be a major determinant of linear growth in young diabetics only during childhood, but not during puberty, where other factors such as sex hormones may play a more important role. The decrease in IGF-I levels seems to be even more marked when freely available IGF-I is considered: since IGF-I levels are low and IGFBP-3 levels elevated in IDDM, the IGF-I-to-IGFBP-3 ratio is markedly reduced. It is tempting to speculate that poor metabolic control may impair linear growth of diabetic children at least partially via depression of circulating IGF-I levels and the alteration of IGFBP serum concentrations. The question whether such changes of IGF and IGFBP levels during childhood and adolescence also might contribute to the development of late diabetic complications needs to be addressed in the future.

References

1. **Flyvbjerg A, Orskov H, Alberti KGMM, eds.** 1993 GH and IGF-I in Human and Experimental Diabetes: Basic and Clinical Aspects. Chichester: John Wiley.
2. **Kiess W, Kessler U, Schmitt S, Funk B.** 1993 GH and IGF-I: basic aspects. In: Flyvbjerg A, Orskov H, Alberti KGMM, eds. GH and IGF-I in Human and Experimental Diabetes: Basic and Clinical Aspects. Chichester: John Wiley, pp 1–23.
3. **Rutanen E-M, Pekonen F.** 1990 Insulin-like growth factors and their binding proteins. Acta Endocrinol (Copenh). 123:7–13.
4. **Holly JMP.** 1993 Insulin-like growth factor binding proteins in diabetic and nondiabetic states. In: Flyvbjerg A, Orskov H, Alberti K, eds. Growth Hormone and Insulin-Like Growth Factor I. Chichester: John Wiley & Sons Ltd., pp 47–56.
5. **Merimee TJ, Zapf J, Froesch R.** 1983 Insulin-like growth factors. Studies in diabetics with and without retinopathy. N Engl J Med. 309:527–530.
6. **Tamborlane WV, Hintz RL, Bergman N, et al.** 1981 Insulin-infusion pump treatment of diabetes. Influence of improved metabolic control on plasma somatomedin levels. N Engl J Med. 305:303–307.
7. **Rechler MM, Nissley SP.** 1990 Insulin-like growth factors. In: Sporn MB, Roberts AB, eds. Peptide Growth Factors and Their Receptors I. Handbook of Pharmacology. Heidleberg: Springer, pp 263–367.
8. **Zapf J, Walters H, Froesch ER.** 1981 Radioimmunological determination of insulin like growth factors I and II in normal subjects and in patients with growth disorders and extra-pancreatic tumor hypoglycemia. J Clin Invest. 68:1321–1330.
9. **Daughaday WH, Trivedi B, Kapadia M.** 1981 Measurement of IGF-II by a specific RRA in serum of normal individuals, patients with abnormal GH secretion and patients with tumor associated hypoglycemia. J Clin Endocrinol Metab. 53:289–294.
10. **Baxter RC.** 1991 Insulin-like growth factor (IGF) binding proteins: the role of serum IGFBPs in regulating IGF availability. Acta Paediatr Scand (Suppl). 372:107–114.
11. **Humbel RE.** 1990 Insulin-like growth factors-I and -II. Eur J Biochem. 190:445–462.
12. **Ritvos O, Ranta T, Jalkanen J.** 1988 Insulin-like growth factor (IGF)-binding protein from human decidua inhibits the binding and biological action of IGF-I cultured choriocarcinoma cells. Endocrinology. 122:2150–2157.
13. **Rutanen E-M, Pekonen, Mäkinen T.** 1988 Soluble 34K binding protein inhibits the binding of insulin-like growth factor I to its cell receptors in human secretory phase endometrium: evidence for autocrine/paracrine regulation of growth factor action. J Clin Endocrinol Metab. 66:173–180.
14. **Holly JMP.** 1991 The physiological role of IGFBP-1. Acta Endocrinol (Copenh). 124:55–62.
15. **De Vroede MA, Tseng LY-H, Katsoyannis PG, et al.** 1986 Modulation of insulin like growth factor I binding to human fibroblast monolayer cultures by insulinlike growth factor carrier proteins released to the incubation media. J Clin Invest. 77:602–613.
16. **Elgin RG, Busby WH, Clemmons DR.** 1987 An insulin-like growth factor (IGF) binding protein enhances the biologic response to IGF-I. Proc Natl Acad Sci USA. 84:3254–3258.
17. **Clemmons DR, Elgin RG, Han VKM, et al.** 1986 Cultured fibroblast monolayers secrete a protein that alters the cellular binding of somatomedin-C/insulin-like growth factor I. J Clin Invest. 77: 1548–1556.
18. **Lewitt MS, Baxter RC.** 1991 Cytochalasin B stimulates insulin-like growth factor-binding protein-1 production by Hep G2 cells. Mol Cell Endocrinol. 77:149–157.
19. **Conover CA, Lee PDK.** 1990 Insulin regulation of insulin-like growth factor-binding protein production in cultured HepG2 cells. J Clin Endocrinol Metab. 70:1062–1067.
20. **Zapf J, Schmid C, Guler HP, et al.** 1990 Regulation of binding proteins for insulin-like growth factors in humans. Increased expression of IGF binding protein 2 during IGF-I treatment of healthy adults and in patients with extrapancreatic tumor hypoglycemia. J Clin Invest. 86:952–961.
21. **Ooi GT, Orlowski CC, Brown AL, Becker RE, Unterman TG, Rechler MM.** 1990 Different tissue distribution and hormonal regulation of mRNAs encoding rat insulin-like growth factor binding proteins-1 and -2. Mol Endocrinol. 4:321–328.
22. **Blum WF, Horn N, Kratzsch J, et al.** 1993 Clinical studies of IGFBP-2 by radioimmunoassay. Growth Regul. 3:100–105.
23. **Clemmons DR, Snyder DK, Busby WH.** 1991 Variables controlling the secretion of insulin-like growth factor binding protein-2 in normal human subjects. J Clin Endocrinol Metab. 73:727–733.
24. **Lewitt MS, Baxter RC.** 1990 Inhibitors of glucose uptake stimulate the production of insulin-like binding protein (IGFBP-1) by human fetal liver Endocrinology. 126:1527–1533.
25. **Holly JMP, Biddlecombe RA, Sandhu RR, et al.** 1988 Circadian variation of insulin like growth factor small molecular weight binding protein (IGF-SBP) in normal adults and adolescents with growth disorders or diabetes mellitus. J Endocrinol [Suppl]. 117:246.
26. **Cotteril AM, Cowell CT, Baxter RC, McNeil D, Silink M.** 1988 Regulation of the growth hormone independent growth factor binding protein in children. J Clin Endocrinol Metab. 67:882–887.
27. **Suikkari A-M, Koivisto VA, Rutanen E-M, et al.** 1988 Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. J Clin Endocrinol Metab. 66:266–272.
28. **Brismar K, Gutniak M, Povoa G, et al.** 1988 Insulin regulates the 35

kDa IGF binding protein in patients with diabetes mellitus. J Endocrinol Invest. 11:599–602.
29. **Blum WF, Hall K, Ranke MB, Wilton P.** 1993 GH insensitivity syndromes: a preliminary report on changes in IGFs and their binding proteins during treatment with recombinant IGF-I. Acta Paediatr Suppl. 391:15–19.
30. **Breier BH, Milsom SR, Blum WF, Schwander J, Gallaher BW, Gluckman PD.** 1993 IGFs and their binding proteins in plasma and milk after GH-stimulated galactopoiesis in normally lactating women. Acta Endocrinol (Copenh). 129:427–435.
31. **Laron Z, Klinger B, Blum WF, Silbergeld A, Ranke MB.** 1992 IGFBP-3 in patients with Laron type dwarfism: effect of exogenous rIGF-I. Clin Endocrinol (Oxf). 36:301–304.
32. **Baxter RC, Cowell CT.** 1987 Diurnal rhythm of growth hormone-independent binding protein for insulin-like growth factors in human plasma. J Clin Endocrinol Metab. 65:432–440.
33. **Crosby SR, Tsigos C, Anderton CD, et al.** 1992 Elevated plasma insulin-like growth factor binding protein-1 levels in type 1 (insulin-dependent) diabetic patients with peripheral neuropathy. Diabetologia. 35:868–872.
34. **Blum WF.** 1992 IGFs and their binding proteins. In: Ranke M, ed. Functional Endocrinologic Diagnostics in Children and Adolescents. Mannheim: J & J Verlag, pp 102–117.
35. **Blum WF, Ranke MB, Bierich JR.** 1988 A specific radioimmunoassay for insulin like growth factor II: the interference of IGF binding proteins can be blocked by excess IGF-I. Acta Endocrinol (Copenh) 118:374–380.
36. **Blum WF, Breier B.** 1994 Radioimmunoassays for IGFs and IGFBPs. Growth Regul. 4 (Suppl 1):11–19.
37. **Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB.** 1993 Serum levels of IGF-I and IGFBP-3 reflect sponaneous GH secretion. J Clin Endocrinol Metab. 76:1610–1616.
38. **Prader A, Largo AH, Molinari L, Issler RH.** 1989 Physical growth in Swiss children from birth to 20 years of age. Helv Paediatr Acta. 52 (Suppl):1.
39. **Amiel SA, Sherwin RS, Hintz RL, et al.** 1984 Effect of diabetes and its control on insulin-like growth factors in the young subject with type I diabetes. Diabetes. 33:1175–1179.
40. **Blethen SL, Sargeant DT, Whiltlow MG, Santiago JV.** 1981 Effect of pubertal stage, and recent blood glucose control on plasma somatomedin C in children with insulin-dependent diabetes mellitus. Diabetes. 30:868–873.
41. **Cacciari E, Salardi S, Ballardini D, et al.** 1985 Plasma somatomedin-C in children and adolescents with insulin-dependent diabetes mellitus (IDDM): relationship to pubertal stage, metabolic control, growth velocity and fluoroangiographic retinal changes. J Pediatr Endocrinol. 1:177–186.
42. **Dunger DB, Holly J, Cheetham T, et al.** 1993 The relationship between GH, IGF-I, IGFBP-3 and GH binding proteins (GHBP) in normal and adolescents with insulin-dependent diabetes mellitus (IDDM). Diabetes. 2 (Suppl 1):691.
43. **Massa G, Dooms L, Bouillon R, Vanderschueren-Lodeweyckx M.** 1993 Serum levels of growth hormone-binding protein and insulin-like growth factor I in children and adolescents with type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 36: 239–243.
44. **Tan K, Baxter R.** 1986 Serum insulin-like growth factor I levels in adult diabetic patients: the effect of age. J Clin Endocrinol Metab. 63:651–655.
45. **Maes M, Underwood LE, Ketelslegers JM.** 1986 Low serum somatomedin-C in insulin-dependent diabetes: evidence for a post-receptor mechanism. Endocrinology. 118:377–382.
46. **Bornfeldt KE, Arnqvist HJ, Enberg B, et al.** 1989 Regulation of insulin-like growth factor-I and growth hormone recptor gene expression by diabetes and nutritional state in rat tissues. J Endocrinol. 122:651–656.
47. **Rogers DG, Sherman LD, Gabbay KH.** 1991 Effect of puberty on insulin-like growth factor I, and HbA1 in type I diabetes. Diabetes Care. 14:1031–1035.
48. **Batch JA, Baxter R, Werther G.** 1991 Abnormal regulation of insulin-like growth factor binding proteins in adolescents with insulin-dependent diabetes. J Clin Endocrinol Metab. 73:964–968.
49. **Menon RK, Arslanian S, May B, et al.** 1992 Diminished growth hormone-binding protein in children with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 74:934–938.
50. **Rosenfield RL, Furlanetto R.** 1985 Physiologic testosterone or estradiol induction of puberty increases plasma somatomedin-C. J Pediatr. 107:415–417.
51. **Jasper HG.** 1985 Somatomedin response to testosterone stimulation in children with male pseudohermaphroditism, cryptorchidism, anorchia, or micropenis. J Clin Endocrinol Metab. 60:910–913.
52. **Rogers DG, Valdes CT, Elkind-Hirsch KE.** 1990 The effect of ovarian function on insulin-like growth factor I plasma levels and hepatic IGF-I mRNA levels in diabetic rats treated with insulin. Diabetes Res Clin Pract. 8:235–242.
53. **Rudolf MCJ, Sherwin RS, Markowitz R, et al.** 1982 Effect of intensive insulin treatment on linear growth in the young diabetic patient. J Pediatr. 101:333–339.
54. **Dacou-Voutetakis C, Dracopoulou M, Georgopoulos N, et al.** Height, IGF I, GH, and HbA1 in children and adolescents with insulin-dependent diabetes mellitus. Presented at the 75th Annual Meeting of The Endocrine Society, Las Vegas, NV, 1993 (Abstract 738c).
55. **Povoa G.** 1987 Studies on somatomedin binding protein. J Endocrinol. Invest. 10 (Suppl 4): Abstract 22.
56. **Brismar K, Hall K.** 1993 Clinical applications of IGFBP-1 and its regulation. Growth Regul. 3:98–100.
57. **Holly JMP, Dunger DB, Edge JA, et al.** 1990 Insulin like growth factor binding protein-1 levels in diabetic adolescents and their relationship to metabolic control. Diabetic Med. 7:618–623.
58. **Taylor AM, Dunger DB, Preece MA, et al.** 1990 The growth hormone independent insulin-like growth factor-I binding protein BP-28 is associated with serum insulin-like growth factor-I inhibitory bioactivity in adolescent insulin-dependent diabetics. Clin Endocrinol (Oxf). 32:229–239.
59. **Baxter RC, Martin JL.** 1986 Radioimmunoassay of growth hormone-dependent insulinlike growth factor binding protein in human plasma. J Clin Invest. 78:1504–1512.
60. **Tattersall RB, Pyke DA.** 1973 Growth in diabetic children: studies in identical twins. Lancet. 2:1105–1109.
61. **Jackson RL.** 1984 Growth and maturation in children with insulin dependent diabetes. Pediatr Clin North Am. 31:545–567.